PATENT COOPERATION TREATY

PCT 10/540759 PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 497047 KXR/akh	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ2003/000294**	International Filing Date *(day/month/year)* 24 December 2003	Priority Date *(day/month/year)* 24 December 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C07K 14/415; C07H 21/04; C12N 15/63, 15/82.

Applicant

THE HORTICULTURE & FOOD RESEARCH INSTITUTE OF NEW ZEALAND LIMITED et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand 21 July 2004	Date of completion of the report 31 March 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **MARIE-ANNE FAM** Telephone No. (02) 6283 2254

INTERNATIONAL PRE[redacted]NARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000294

I. Basis of the report

1. With regard to the **elements** of the international application:*

[ ] the international application as originally filed.

[X] the description, pages **1-51,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[X] the claims, pages **54, 57,** as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **52, 53 and 55,** received on **23 March 2005** with the letter of **23 March 2005** and page **56** received on **14 January 2005** with the letter of **14 January 2005**

[X] the drawings, pages **1/22-22/22,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[X] the sequence listing part of the description:
pages **1-16,** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000294

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-44	YES
	Claims -	NO
Inventive step (IS)	Claims 1-44	YES
	Claims -	NO
Industrial applicability (IA)	Claims 1-44	YES
	Claims -	NO

2. Citations and explanations (Rule 70.7)

Novelty and Inventive Step

The following documents were identified in the International Search Report:

D1 Accession Number AY561842

D2 Accession Number AY561843

D3 Plant Cell

D4 Accession Number AF282875

D1 and D2 were published after the international filing date and hence are excluded from consideration during international preliminary examination.

The present invention relates to the enzyme multifunctional germacrene-D-synthase and its use in the production of sesquiterpenes.

Neither D3 nor D4 describes a multifunctional germacrene-D synthase as defined by the present Sequence Id. No. 1 or Sequence Id. No. 2. Claims 1-44 are therefore considered novel and inventive over the prior art.

Industrial Applicability

Claims 1-44 meet the requirements for industrial applicability.

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Received 23 March 2005

CLAIMS

1. An isolated polynucleotide encoding a multifunctional germacrene-D synthase, wherein the synthase comprises an amino acid sequence with at least 60% similarity to SEQ ID NO:2.

2. An isolated polynucleotide having the sequence of SEQ ID NO:1 or a fragment or variant thereof encoding a polypeptide with multifunctional germacrene-D synthase activity.

3. A polynucleotide as claimed in claim 1 or claim 2 wherein the polynucleotide is capable of facilitating the conversion of FDP to a mixture of germacrene-D and one or more other sesquiterpenes selected from *delta*-cadinene, *delta*-elemene, elemol, *gamma*-muurolene, *gamma*-cadinene, *gamma*-elemene and germacrene B.

4. An isolated polynucleotide as claimed in claim 3 wherein the sequence has at least 60% identity to the nucleotide sequence of SEQ ID NO:1.

5. An isolated polynucleotide as claimed in claim 3 wherein the sequence has at least 90% identity to the nucleotide sequence of SEQ ID NO:1.

6. An isolated polynucleotide as claimed in claim 3 wherein the sequence has at least 95% identity to the nucleotide sequence of SEQ ID NO:1.

7. An isolated polynucleotide as claimed in claim 3 wherein the nucleotide sequence is that of SEQ ID NO:1.

8. An isolated polynucleotide encoding the polypeptide of SEQ ID NO:2 or encoding a variant or a fragment of that sequence which has a multifunctional germacrene-D synthase activity.

9. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has at least 60% identity with the amino acid sequence of SEQ ID NO:2.

10. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has at least 90% identity with the amino acid sequence of SEQ ID NO:2.

11. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has at least 95% identity with the amino acid sequence of SEQ ID NO:2.

12. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has the sequence of SEQ ID NO:2.

13. An isolated multifunctional germacrene-D synthase polypeptide comprising an amino acid sequence with at least 60% similarity to SEQ ID NO:2.

14. An isolated multifunctional germacrene-D synthase having the sequence of SEQ ID NO:2 or a fragment or variant thereof with multifunctional germacrene-D synthase activity.

15. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence has at least 60% identity with the sequence of SEQ ID NO:2.

16. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence has at least 90% identity with the sequence of SEQ ID NO:2.

17. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence has at least 95% identity with the sequence of SEQ ID NO:2.

18. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence is a mature sequence derived from SEQ ID NO:2.

19. A genetic construct comprising a polynucleotide of any one of claims 1 to 12.

31. A transgenic plant comprising a plant cell as claimed in claim 30.

32. A method of preparing germacrene-D, *delta*-cadinene, *gamma*-cadinene, *gamma*-muurolene, *gamma*-elemene, *delta*-elemene, elemol or germacrene B comprising the steps of

(a) culturing a cell which has been genetically modified with a polynucleotide any one of claims 1-12 to provide increased multifunctional germacrene-D synthase activity;

(b) providing the cell with farnesyl diphosphate or geranyl diphosphate if necessary; and

(c) separating the germacrene-D and/or *delta*-cadinene and/or *delta* elemene and/or elemol and/or germacrene B, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene produced.

33. A method for modulating the Germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene production of a plant, the method comprising: increasing or decreasing expression of multifunctional germacrene-D synthase wherein said increasing or decreasing is achieved by genetic modification to alter the expression of a gene encoding a multifunctional germacrene-D synthase, wherein the synthase comprises an amino acid sequence with at least 60% similarity to SEQ ID NO:2.

34. A method as claimed in claim 33 wherein the synthase comprises a synthase with the sequence of SEQ ID NO: 2.

35. A method for modulating germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene production in a plant, the method comprising of :

(a) introducing into the plant, a genetic construct of claims 19-27; and

(b) transcriptionally expressing the polynucleotide in the plant.

36. A method for modulating germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene production in a plant, the method comprising of

(a) introducing into the plant, a DNA genetic construct of claims 19-27; and

(b) expressing the polypeptide in the plant.

37. A polynucleotide fragment of SEQ ID NO:1 comprising at least 15 contiguous nucleotides.

38. A method of selecting a plant with altered germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene content comprising the steps of:

(a) contacting polynucleotides from at least one plant with at least one polynucleotide comprising at least 15 contiguous nucleotides of the polynucleotide of claim 1 to assess the expression of multifunctional germacrene-D synthase; and

(b) selecting a plant showing altered expression.

39. A method as claimed in claim 38 wherein the polynucleotide has at least 15 contiguous nucleotides from a sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 7 and the plant is a plant of the genus *Actinidia*.

40. A method as claimed in claim 38 wherein the plant is a plant of the genus *Vaccinium*.

41. A method for preparing a sesquiterpene comprising:

(a) obtaining a polypeptide as claimed in any one of claims 13-18; and

(b) incubating farnesyl diphosphate in the presence of the polypeptide, and

(c) separating the germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene produced.